AccuDiag™ Legionella Total (IgG/IgM/IgA) ELISA Kit

REF 1651-P1

IVD See External Label 2°C 8°C 96 Tests

Legionella Total (IgG/IgM/IgA) ELISA	
Principle	Indirect ELISA
Detection	Qualitative
Sample	10 µL serum/plasma
Incubation Time	65 minutes
Sensitivity	92.3%
Specificity	93.1%
Shelf Life	12 Months from the manufacturing date

PRODUCT FEATURES

- Very easy to use with little training
- Highly specific and consistent Assay
- Provides accurate results quickly
- Reading of results both visually and as absorbance data

INTENDED USE

The Diagnostic Automation Inc. (DAI) Legionella Total (IgG/IgM/IgA) ELISA Test System is an enzyme-linked immunosorbent assay for the qualitative detection of total antibody (IgG/IgM/IgA) to *Legionella pneumophila* serogroups 1-6 in human sera. This device is for *in vitro* diagnostic use.

SIGNIFICANCE AND SUMMARY

Scientists identified *L. pneumophila* as the causative agent for Legionellosis (Legionella pneumonia, or Legionnaire's Disease) in 1977 (1). Presently, there are more than 25 species and 33 serogroups in the family *Legionellaceae*, with at least 18 species associated with pneumonia, accounting for roughly 1-5% of all cases of pneumonia (2). *L. pneumophila* displays a multitude of morphologies including the bacillus, coccobacillus, and elongated fusiform. Although often difficult to perform, the Gram stain will be Gram-negative.

The antibody response to *L. pneumophila* may be both specific and nonspecific, since the patient may have antibodies to similar antigens from other Gram-negative bacteria. Optimum times for specimen collection appear to be within the first week of illness, or as soon as possible after the onset (acute specimen), and at least 3 weeks after the onset (convalescent specimen) (3). By the IFA method, a single result of ≥1:256 is considered presumptive evidence of legionella infection. Diagnostic titers have been reported to be absent in as many as 25% of patients (4), but the use of multiple Legionella species (5,6) as the antigen source and a polyvalent conjugate directed against IgG, IgM, and IgA (7) maximize the accuracy of serological procedures.

ASSAY PRINCIPLE

The DAI Legionella Total (IgG/IgM/IgA) ELISA test system is designed to detect antibodies to *L. pneumophila* in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive adsorption with Legionella antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG/IgA/IgM is added to the wells and the plate is incubated. The Conjugate will react with the antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.
3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

SPECIMEN COLLECTION & PREPARATION

1. It is recommended that the user carry out specimen collection in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay. No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2° and 8°C for no longer than 48 hours. If delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (14).

REAGENTS

Each kit contains the following components in sufficient quantities to perform the number of tests indicated on packaging label. **Note: All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% (w/v): Controls, Calibrator, and Sample Diluent.**

Materials provided with the kit

1. **Plate:** 96 wells configured in twelve 1x8-well strips coated with a formalin-inactivated sonicated preparation of L. pneumophila Groups 1-6 antigens. The strips are packaged in a strip holder and sealed in an envelope with desiccant.
2. **Conjugate:** Conjugated (horseradish peroxidase) goat anti-human IgG/ IgA/IgM. Ready to use. One, 15 mL vial with a white cap. Ready to use.
3. **Positive Control** (Human Serum): One, 0.35 mL vial with a red cap.
4. **Calibrator** (Human Serum): One, 0.5 mL vial with a blue cap.
5. **Negative Control** (Human Serum): One, 0.35 mL vial with a green cap.
6. **Sample Diluent**. One 30 mL bottle (green cap) containing Tween-20, bovine serum albumin and phosphate- buffered-saline. Ready to use. **Note: The Sample Diluent will change color when combined with serum.**
7. **TMB:** One 15 mL amber bottle (amber cap) containing 3,3',5,5'-tetramethylbenzidine (TMB). Ready to use.
8. **Stop solution:** One, 15 mL, red capped, bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
9. **Wash buffer concentrate (10X):** Dilute 1-part concentrate + 9 parts deionized or distilled water. One, 100 mL, clear-capped, bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTES: The DAI Legionella Total (IgG/IgM/IgA) ELISA test system also contains a component list containing lot specific information inside the kit box, and a Package insert providing instructions for use.

Materials required but not provided

1. ELISA microwell reader capable of reading at a wavelength of 450nm. *NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.*
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering (50-200µL).
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One-liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.
10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant. (i.e.: 10% household bleach, 0.5% sodium hypochlorite.)

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20-25°C).
2. Determine the number of microwells needed. Allow six Control/Calibrator determinations (one Blank, one Negative Control, three Calibrators and one Positive Control) per run. A Reagent Blank should be run on each assay. Check software and reader requirements for the correct Controls/ Calibrator Configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2°and 8°C.

EXAMPLE PLATE SET-UP

	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum. **Note: The Sample Diluent will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted control, calibrator and sample. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of Sample Diluent to well A1 as a reagent blank. Check software and reader requirements for the correct reagent blank well configuration.
6. Incubate the plate at room temperature (20-25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure:**
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps a. and b. for a total of 5 washes.
      4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.

   b. **Automated Wash Procedure:**
      If using an automated microwell wash system, set the dispensing volume to 300-350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.
8. Add 100µL of the Conjugate to each well, including reagent blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20-25°C) for 25 + 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including reagent blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20-25°C) for 10 to 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including reagent blank well, at the same rate and in the same order as the TMB was added. Positive samples will turn from blue to yellow. After adding the Stop
    Solution, tap the plate several times to ensure that the samples are thoroughly mixed.

14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the reagent blank. Read the plate within 30 minutes after the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell – 100µL/well.
3. → *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate – 100µL/well.
6. → *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB – 100µL/well.
9. → *Incubate 10 - 15 minutes.*
10. Add Stop Solution – 50µl/well – Mix.
11. READ within 30 minutes.

RESULTS

1. **Calculations**
   a. *Correction Factor:* The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. The correction factor is determined for each lot of kit components and is printed on the Component Label located in the kit box.

b. *Cutoff OD Value:* To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above.
(CF x Mean OD of Calibrator = Cutoff OD Value)

c. *Index Values/OD Ratios:* Calculate the Index Value/OD Ratio for each specimen by dividing its OD value by the Cutoff OD from step b.

Example:	
Mean OD of Calibrator	0.793
Correction Factor (CF)	0.25
Cut off OD	0.793 x 0.25 = 0.198
Unknown Specimen OD	0.432
Specimen Index Value or OD Ratio	0.432 / 0.198 = 2.18

INTERPRETATION

1. **Interpretations:** Index Values/OD ratios are interpreted as follows:

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

   a. An OD ratio ≤0.90 indicates no significant amount of IgG/IgM/IgA antibodies to *L. pneumophila* detected. A non-reactive result may be equivalent to an IFA titer of less than 1:256. A negative result does not exclude Legionella infection.
   b. An OD ratio ≥1.10 indicates that IgG/IgM/IgA antibodies specific to *L. pneumophila* were detected and is suggestive of Legionella infection at some time, and may be equivalent to an IFA titer of greater than or equal to 1:256. Other laboratory procedures or additional clinical information may be necessary to establish a diagnosis.
   c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal using an alternate serological method and/or re-evaluate by drawing another sample one to three weeks later.

NOTE: The magnitude of the measured result above the cut-off is not indicative of the total amount of antibody present and cannot be correlated of IFA titers.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator and the OD values for the Positive and Negative Controls should fall within the following ranges:

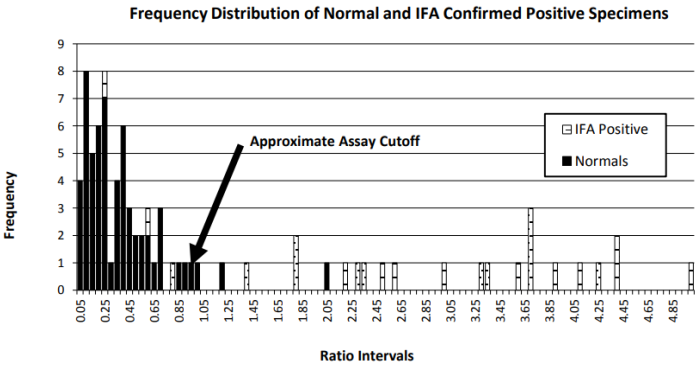
	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥ 0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤ 0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure and will not ensure precision at the assay cut-off.
5. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.

Refer to NCCLS document C24: Statistical Quality Control for Quantitative Measurements for guidance on appropriate QC practices

EXPECTED RANGES OF VALUES

Some researchers have reported background frequencies of elevated antibody levels in a normal population of 1 to 3% for formalin fixed antigen preparations (11). In an evaluation of sixty normal donor sera conducted in-house, one specimen was equivocal (1.7%), two specimens were positive (3.3%), and the remainder (57/60 or 95%) were negative. Below appears a Frequency Distribution of the results of a group of 60 normal donor specimens, and 24 IFA confirmed positive specimens.



PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

Performance of a comparative study demonstrated the substantial equivalence of the DAI ELISA Legionella Total (IgG/IgM/IgA) Test System to another commercially available ELISA test system and to an IFA Legionella Test System. A three-site clinical investigation evaluated the performance of the DAI ELISA Legionella Total (IgG/IgM/IgA) Test System. One clinical site compared the performance of the DAI ELISA Legionella Total (IgG/IgM/IgA) Test System to another commercially available ELISA test system. A second clinical site compared the DAI ELISA Legionella Total (IgG/IgM/IgA) Test System to the DAI IFA Legionella Test System. The third clinical site compared the DAI ELISA Legionella Total (IgG/IgM/IgA) Test System to a commercially available IFA Legionella Test System. The study tested a total of 240 specimens. Clinical specimens tested at sites one and two consisted primarily of routine specimens from a reference laboratory in Northeastern United States that tested for normal Legionella serological analysis. Some repository specimens were included which had been previously tested and were found to be positive for antibody to Legionella. Specimens tested at the third clinical site consisted of 22 paired specimens (acute and convalescent) from confirmed cases of Legionella infection. Tables 1, 2, and 3 show a summary of these comparative investigations. Analysis excluded any equivocal specimens.

Table 1: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site One.

		DAI Legionella ELISA Test System			
		Positive	**Negative**	**Equivocal**	**Total**
Commercial ELISA Test System	**Positive**	12	1	2	15
	Negative	5	67	10	82
	Equivocal	11	0	1	12
	Totals	28	68	13	109

Relative Sensitivity = 12/13 = 92.3% (95% Confidence Interval* = 77.8 to 100%)
Relative Specificity = 67/72 = 93.1% (95% Confidence Interval* = 87.2 to 98.9%)
Relative Agreement = 79/85 = 92.9% (95% Confidence Interval* = 85.7 to 98.4%)
*95% confidence intervals calculated using the exact method.

Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement; Study Site Two.

		DAI Legionella ELISA Test System			
		Negative	**Equivocal**	**Positive**	**Total**
DAI Legionella IFA Test System	**< 1:128**	56	2	7	65
	1:128	0	1	4	5
	≥ 1:256	1	0	16	17
	Totals:	57	3	27	87

Relative Sensitivity = 16/17 = 94.1% (95% Confidence Interval* = 82.9 to 100%)
Relative Specificity = 56/63 = 88.9% (95% Confidence Interval* = 81.1 to 96.6%)
Relative Agreement = 72/80 = 90.0% (95% Confidence Interval* = 83.4 to 96.6%)
* 95% confidence intervals calculated using the exact method.

Table 3: Calculation of Relative Sensitivity, Specificity and Agreement; Study Site Three. (Individual results for testing acute and convalescent specimens)

		DAI Legionella ELISA Test System			
		Negative	**Equivocal**	**Positive**	**Total**
DAI Legionella IFA Test System	**<1:128**	16	0	1	17
	1:128	3	1	1	5
	≥1:256	3	0	19	22
	Totals:	22	1	21	44

Relative Sensitivity = 19/22 = 86.4% (95% Confidence Interval* = 72 to 100%)
Relative Specificity = 16/17 = 94.1% (95% Confidence Interval* = 89.2 to 100%)
Relative Agreement = 35/39 = 89.7% (95% Confidence Interval* = 80.2 to 99.2%)
* 95% confidence intervals calculated using the exact method.

With respect to Table 3 above; of the 22 pairs of acute and convalescent specimens, 17/22 were ELISA negative for the acute, and positive for the convalescent. Of the remaining five pairs 3/22 were negative for both acute and convalescent, and 2/22 were positive for both the acute and convalescent.
NOTE: Be advised that relative refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence.

2. **Precision and Reproducibility:**

To demonstrate inter-laboratory reproducibility of the assay outcome, six specimens were evaluated; Two with an IFA titer of <1:128, 2 with an IFA titer of 1:512, and 2 with an IFA titer of ≥1:1024. Five vials of each specimen were prepared for a total of 30 vials. The 30 vials were randomized and simply numbered 1 through 30. The panel was tested in-house and at the two clinical sites. The study demonstrated excellent inter-laboratory reproducibility, with 100% agreement between all three sites. Precision was evaluated as outlined in CLSI/NCCLS document number EP5-T2: Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition. Both clinical sites performed reproducibility studies using the same eight specimens: two relatively strong positive specimens, two specimens near the cutoff, two that were clearly negative and the kit's negative control and positive control. On each day of testing, each specimen was assayed in duplicate, at two time points, morning and afternoon, for a total of four replicates per specimen. This reproducibility study ran for a 20-day period, for a total of 80 observations, for each of the eight panel members. A summary appears in Table 4 below:

Table 4: Summary of Precision Testing Conducted at Clinical Sites 1 and 2

Specimen	Site	Mean Ratio	Result	SWR*	ST**	Days	Total Observations	Overall % CV
L-1	1	2.264	Positive	0.204	0.249	19	76	10.99
	2	2.517		0.138	0.438	19	76	17.42
L-2	1	2.277	Positive	0.101	0.209	18	72	9.20
	2	2.435		0.123	0.357	20	80	14.67
L-3	1	0.479	Negative	0.024	0.040	18	72	8.45
	2	0.245		0.023	0.049	20	80	19.91
L-4	1	0.281	Negative	0.013	0.032	19	76	11.24
	2	0.077		0.020	0.027	20	80	35.33
L-5	1	1.055	Near	0.081	0.199	19	76	11.32
	2	0.757	Cut-off	0.049	0.091	20	80	12.07
L-6	1	0.845	Near	0.033	0.079	19	76	9.36
	2	0.606	Cut-off	0.060	0.095	20	80	15.72
Positive Control	1	6.414	Positive	0.114	0.297	20	80	4.64

Specimen	Site	Mean Ratio	Result	SWR*	ST**	Days	Total Observations	Overall % CV
Negative Control	1	0.270	Negative	0.019	0.033	20	80	12.14

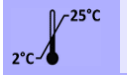
*Point estimate of within run precision standard deviation.
**Point estimate of total precision standard deviation

NOTE: Table 4 depicts the reproducibility results only as an example of those results obtained during the clinical study, using ideal conditions of environment, equipment, and technique. Each laboratory should evaluate reproducibility as it may vary depending upon the conditions at the laboratory.

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on anti-Legionella results alone. Test results for anti-Legionella should be interpreted in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. A positive result suggests infection with one or more of the Group 1-6 species; however, one will not be able to distinguish between species with the results of this ELISA test alone.
3. Avoid the use of hemolytic, lipemic, bacterially contaminated or heat inactivated specimens. Erroneous results may occur.
4. Cross-reactivity may occur in sera with infections due to other Legionella species.
5. A negative result does not rule out the possibility of infection with legionella. Serum specimens taken too early during the course of infection may not yet have significant antibody titers. Some culture positive cases of Legionella do not develop antibody to Legionella.
6. Positive results may be due to cross reactivity with antibody generated as a result of non-Legionella infection. Serologic cross-reactions have been reported with *P. aeruginosa,* several Rickettsia species, *Coxiella burnetii*, enteric gram-negative rods, *Bacteroides* species, *Haemophilus* species, *Citrobacter freundii*, and *Campylobacter jejuni*. Therefore, a positive result alone does not indicate infection with Legionella. Additionally, some reports indicate that a number of apparently healthy individuals may carry antibodies to legionellae; however, a positive result, along with clinical signs and symptoms may indicate possible Legionella infection. Additional serologic testing, such as paired sera analysis by IFA, or other clinical testing such as direct FA and culturing, may be necessary to establish diagnosis.
7. Assay performance characteristics for matrices other than sera have not been established.
8. The affinity and/or avidity of the anti-IgG/IgM/IgA conjugate has not been determined.
9. Although the conjugate is designed to detect human IgG, IgM, and/or IgA, one will not be able to determine which antibody is present with this assay.
10. Early antibiotic therapy may suppress antibody response and some individuals may not develop antibodies above detectable limits.
11. A single positive result only indicates previous immunologic exposure; level of antibody response may not be used to determine active infection.
12. Use of serogroups 1-6 for assessing antibody responses to different Legionella species and serogroups has not been established. Some infected patients may not have detectable levels of antibodies with this assay. Four to eight weeks may be needed to detect an antibody response and antibody levels can fall to undetectable levels within a month of infection.

STORAGE CONDITIONS

	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening - strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent
	Stop Solution: 2 - 25°C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days. Wash Buffer (10X): 2 - 25°C

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (13).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The Sample Diluent, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions upon hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.

14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.
22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

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MANUFACTURER AND BRAND DETAILS

ISO 13485:2016

bsi ISO 13485 Quality Management for Medical Devices CERTIFIED

Diagnostic Automation/Cortez Diagnostics, Inc.
21250 Califa Street, Suite 102 and 116,
Woodland Hills, California 91367 USA

Date Adopted	**2023-09**
Brand Name	**AccuDiag™**
REF 1651-P1	**AccuDiag™ - Legionella Total (IgG/IgM/IgA) ELISA**
EC REP	**CEpartner4U, Esdoornlaan 13, 3951 DB Maarn, The Netherlands www.cepartner4u.eu**
Revision Date: 2017-12-19	